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No. 6.—ON THE DIPLOCOCCOID FORM OF THE
COLON BACILLUS.

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WITH THREE PLATES.

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ON THE DIPLOCOCCOID FORM OF THE COLON BACILLUS.

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In the course of a careful study of a long series of livers, both cirrhotic and otherwise, we have in the specimens examined, with scarce an exception, encountered larger or smaller numbers of minute bodies, and the more we have studied them the more assured we have become that these are bacterial in nature. Under the ordinary $\frac{1}{2}$ immersion lens, and by the usual methods of staining these may easily be overlooked, and if recognized they may easily be mistaken for minute pigment granules present in the liver cells. But by more intensive staining and by employing a good $\frac{1}{8}$ immersion lens their nature becomes more evident.

The methods we have employed with the greatest amount of success have been by staining with carbol-fuchsin (one-half the ordinary strength) and subsequent bleaching in the sunlight in our earlier observations, and of late, almost exclusively, carbol-thionin, made according to the formula in Muir and Ritchie's text-book, the sections being cleared by aniline oil. Stained by either of these methods the granules resolve themselves in the main into the fine diplococci, surrounded often by a fine halo, as to the nature of which we shall speak later. When these diplococci are present in any numbers there may also be isolated minute spherical and ovoid bodies of the same dimensions, and there may also be seen occasional strings of three or four coccus-like bodies.

We have recognized these in the livers of man, the cow, sheep, rabbit, and guinea-pig. At first, working with the cirrhotic livers

of cattle and man, one of us was inclined to regard these as peculiar to cirrhosis, but, as already announced,¹ fuller study having shown their existence in the apparently normal liver, they cannot be regarded as specific of any one disease, although it is possible that they are one factor in the production of certain forms of fibrosis. Under these conditions they tend to take on a relatively deep stain, but in the majority of cases they stain badly, have a characteristic brownish tinge and would seem to be dead.

From several cases of cirrhosis in which these were recognizable cultures gave either vigorous, or what we must now regard as attenuated, growths of a colon bacillus, while after intravenous inoculation of adult rabbits with forty-eight-hour broth growths of our stock culture of the colon bacillus, which is in every respect typical, the liver cells showed these minute diplococcus forms in enormous numbers.

We were, therefore, led to conclude that, while it might be that other bacillary forms may also show a diplococcus-like appearance in the tissues, we had adequate evidence that the colon bacillus can show this appearance, and during the last few months we have conducted a long series of observations bearing more especially upon this diplococcus-like modification of the bacillus. Our work is divisible into two portions:

I. On the production of a diplococcoid form of the colon bacillus outside the organism.

II. On the diplococcoid form of the bacillus within the tissues.

The former portion has been undertaken in part by Dr. Nicholson; the latter portion and the studies upon growths in body fluids by Dr. Maude E. Abbott.*

PART I.

ON THE PRODUCTION OUTSIDE THE BODY OF A DIPLOCOCCOID FORM OF THE COLON BACILLUS.

While, under ordinary conditions of growth outside the body and ordinary staining by Loeffler's blue, for example, the colon is an

* But for the fact that, by my previous publication on the subject, I have made myself peculiarly responsible for these observations upon the colon bacillus, I would very gladly have left my own name off the title-page, for, in consequence of prolonged absence from my laboratories, the observations have been throughout conducted by Dr. Abbott, and Mr., now Dr. Nicholson, and I cannot sufficiently acknowledge the enthusiasm which they have thrown into the work.—J. G. A.

undoubted bacillus with no recognizable internal organization, it has been a matter of frequent observation that it might present distinct polar staining, and, indeed, when stained by fuchsin or other strong reagent for purposes of photography this so-called polar staining is very conspicuous. We need but refer to the various published photographs to confirm this statement. In these photographic reproductions of film preparations from cultures the majority of the bacilli are seen to be present as two rounded coccus-like bodies lying in close apposition, a common enclosing or joining sheath being more or less clearly evident.

The appearance here observed is that which is generally spoken of as "polar staining." It is common to a large number of bacteria, and, in not a few cases, as, for example, among the bacteria of hæmorrhagic septicæmia, has led in the past to not a little confusion in descriptions, authorities having been divided as to whether to class bacteria exhibiting the property in a marked degree as bacilli or diplococci. In certain cases, as in connection with the typhoid bacillus, it has been attributed to a retraction of the protoplasm to the poles during the process of preparation and staining of the film of bacteria, and thus has been regarded as an artefact.

We shall not here enter into the discussion concerning polar and metachromatic granules, but simply state that our observations, so far as they go, would seem to negative this latter supposition and to render it evident that, in the case of the colon bacillus at least, *there is a structural condition or internal organization of the microbe underlying and explaining such polar staining.* What is more, they show us that the appearances seen in the colon bacillus are closely allied to the "beading" to be made out in the tubercle bacillus under certain conditions of growth and environment.

As already pointed out by A. Schmidt,² Rodet,³ and others, the colon bacillus varies according to the length of time it is kept outside the body, according to the medium in which it is grown, the reaction of the medium, the temperature, and the part from which it has been isolated. Rodet has found that when it is taken from the healthy intestine the individuals during the earlier generations outside the body are singularly even in their length and thickness and stain well throughout; when taken from diseased tissues, from the inflamed gall-bladder, for example, this is no longer the case; they are irregu-

lar, both in length and thickness; they stain irregularly, and show clear spaces and deeper staining portions.

He points out that a temperature of 44° to 45° leads, during the first few hours, to the appearance of very long filaments, though other individual forms are of the normal length. All these filaments show refractive bodies which take up intensely the basic aniline color. After twenty-four hours these long filaments disappear. In addition, according to this author, growth upon broth containing 2.5 per cent. lactose leads to the peculiar short and small forms, almost like cocci, the majority of which are double and in the form of diplococci.

These observations of Rodet have just come into our hands, and we can in the main confirm them. Indeed, in ignorance of this work, published two years ago, we have been working very much along the same lines as those indicated by Rodet, who, however, it may be added, has noted these appearances without studying more fully their nature.

We find that the long filaments mentioned by Rodet are to be observed in cultures kept for a few hours at a high temperature. It must not, however, be thought that they are exclusively confined to this period. Similar long filaments, showing even more clearly the presence of deeper staining bodies within them, are to be gained from old cultures associated with involution forms. Thus, in a specimen of our stock colon bacillus grown for a fortnight in broth containing a trace of bile we found great numbers of these long bodies, and associated with them numerous small diplococcoid forms. Perhaps the most interesting of these long filaments were observed in an agar plate culture obtained from the liver in a case of cirrhosis, which had been subjected for a few hours to a temperature of about 45° or 46° ; removed from the incubator, this had grown, under difficulties (brought about by the partial drying-up of the medium), for four days at the ordinary temperature. In this the disposition of the deeper staining points was remarkable. (*Vide* Fig. 8, Plate I.) Seen under the $\frac{1}{18}$ immersion lens, after staining by carbol-fuchsin and decolorizing by weak acetic acid, these fine deeper staining points were arranged in a succession of pairs with occasional larger single ovoid bodies interposed. We have come across one other specimen of a rather prolonged growth, in which the same appearance was recognizable, though not quite so clearly. Possibly the exact extent of the staining and subsequent decolorization may have something to do with the diffi-

culty in recognizing this particular arrangement of the contained bodies.

We have also found that taking saliva, filtering and sterilizing it, and making cultures in this medium at the ordinary temperature, we obtain the production of these long filaments, which may be present in the growth not only during the first twenty-four hours, but during the continuance of the culture.

Under these conditions in the saliva of one of us (F. J. N.) the bacilli were throughout singularly slim, and in the later growths again they tended to show the development within the bodies of the bacilli of a succession of deeply-staining dots.

A. Schmidt has noted that he obtained these filamentous forms of the colon bacillus by the addition of caustic soda to broth. We found that we obtained the longest forms by employing lactose broth rendered 1.5° acid to phenolphthallein and containing 2.5 per cent. lactose. Here, more especially on the surface exposed to the air, at the end of twenty-four hours, we obtained remarkably long filaments. Indeed, we cannot agree with Rodet that the addition of this relatively large percentage of lactose to broth results in the production of the diplococcus forms. It is a misfortune that Rodet did not state more precisely the composition and the reaction of his broth.

In order to obtain the diplococcus form of the bacillus we conducted a series of experiments upon growth in broth of varying degrees of alkalinity and acidity at a temperature of 46° . Under these conditions it seemed certain that after the first twenty-four hours we obtained, more especially in slightly acid broths, a relatively increased proportion of short forms with polar staining, but we could not convert all the bacilli into the diplococcoid form. It was when we attempted to grow the bacillus upon certain of the body fluids that we met with the greatest amount of success.

The frequency with which we had encountered this diplococcus form in our observations on the liver made us wonder whether our method of gaining cultures might not have been, in part at least, accountable for the phenomenon. As Livingood¹ has shown, growth of the colon bacillus in organic juices expressed from the liver, spleen, etc., has some slight effect upon the morphology of this microbe. He noted that while the colon bacillus, in general, was relatively very large when grown upon *heated* liver juice, upon *unheated* he obtained very short,

thick, almost oval forms with abrupt ends, these occurring occasionally in pairs. Here in the development of these oval forms there is, it may be urged, an approach toward our diplococcoid form, but so careful an observer would have made a fuller note upon the subject had he recognized constantly the development of the diplococcoid appearance.

But it must be pointed out that there is a difference between inoculating a medium with a loopful of a culture—*i. e.*, with hundreds of thousands of a micro-organism, and employment of a medium in which what bacilli are present have gained an entrance through the ducts and excretory channels of the organs from which the fluid has been obtained. Working with bile, for example, we have frequently found that by making ordinary streak cultures in the usual method we obtained no results, whereas gaining the bile direct from the bladder by means of a pipette and adding a drop or two of this to broth, growths were obtainable.

The conclusion which we have reached is that in such cases the bacteria have been present in relatively small numbers, numbers so small that the somewhat weak inhibitory action of the bile has been sufficient to prevent growth when this bile has not been diluted. All our work goes to show, in fact, that bile has a slight inhibitory effect, not necessarily destroying the micro-organisms, but permitting growth to continue under unfavorable conditions, and it is under these unfavorable conditions that we have obtained either absence of growth or development of the diplococcoid form. For example, we have noticed in several cases that whereas with bile taken immediately from the body we have obtained no cultures, when a pipette of that bile has been kept for several days in the incubator, fairly numerous fine colonies of the *bacillus coli* have developed, in which the individuals show a tendency to assume the diplococcus form. These observations prepared us to find that the diplococcoid form of the *bacillus* might be a modification brought about by the action of the body fluids; but more especially were we led to employ these body fluids by two interesting observations.

In September, 1898, our attention was called by Dr. W. F. Hamilton to a case of what was diagnosed as atrophic cirrhosis in the medical wards of the Royal Victoria Hospital: this diagnosis was subsequently fully confirmed at autopsy. Through the kindly inter-

est of Dr. Hamilton we were present at the first tapping of the patient, and then obtained under careful antiseptic precautions sterilized flasks of ascitic fluid; at the same time a guinea-pig was inoculated with 10 c.c. of the same fluid, and cultures were made directly upon broth, agar, and blood-serum.

A full account of this case is on the point of publication by one of us, and we will here only give a brief epitome of the results.

Upon agar and Loeffler's blood-serum there developed scattered small colonies of a form which at first was taken to be a diplococcus, but which later, in the course of forty-eight hours, upon these media, as in the broth, showed the presence of definite stumpy bacilli, often arranged as short diplo-bacilli—in fact, the form which we recognize as very characteristic of the colon bacillus. Unfortunately, vacation time came on and the opportunity to fully examine these forms passed by. However, the guinea-pig died in twenty-four days, the autopsy was performed a few minutes after death, and from all the organs we obtained a pure culture of the colon bacillus, which appeared to be quite typical. Among the organs from which cultures were made was the gall-bladder; this gave a pure culture of the colon bacillus.

A pipette full of the bile of this guinea-pig, which possessed the characters dwelt upon by Welch and Blachstein⁵—*i. e.*, was clear, abundant, and of a relatively light color—showed, even when placed in the incubator, no apparent growth, but remained unclouded; at most, a few fine, granular flocculi were present after some days. But upon examining a film of this bile which had thus been kept, it was found to contain abundant minute diplococci. (*Vide* Fig. 12, Plate I.) These grew easily when transferred to agar, the colonies being minute and much smaller than those of the typical colon bacillus.

In the first transfer upon broth coccus and diplococcoid forms predominated, with occasional homogeneous stumpy bacilli. (*Vide* Fig. 1, Plate I.) Later transfers upon agar from this broth led to the development of the typical bacillary form—stumpy bacilli with rounded ends, often arranged as short diplo-bacilli, and showing a tendency toward polar staining. The morphological features of the cultures now became coarser, and resembled those of the ordinary colon bacillus.

Evidently, therefore, the bile of the guinea-pig exercised an inhibitory effect upon the growth of the colon bacillus, and this in two

directions. In the first place, the growth was peculiarly slow, so that the bile did not become turbid; in the second, the individual bacilli were distinctly modified—they were very much smaller than normal, and stained in such a way that they might easily be mistaken for minute diplococci. In fact, the resemblance between these minute diplococci and the minute diplococcus forms seen both in the cirrhotic and the normal liver is most striking.

What is true of the bile would seem equally true of the ascitic fluid taken from this case of cirrhosis. The fluid obtained was slightly opalescent, and upon keeping there gradually separated out a thin gelatinous proteid precipitate. Placed in the incubator, the fluid remained clear, and for the first few days appeared to be sterile; by the end of a fortnight, however, a granular deposit was distinguished, and now examination of the fluid showed the presence in it of singularly minute diplococci tending to be arranged in chains. (*Vide* Fig. 2, Plate I.) It may be remarked that this chain-like arrangement of the colon bacillus has been previously observed by Dunbar,⁶ A. Schmidt,² and other workers.

Between the 3d of September and the 13th of October no less than five tapings were made, of which the third and fifth were subjected to examination. Both of these gave cultures upon broth and agar, showing diplococci merging into stumpy ovoid forms. Here, again, cultures when made immediately from the ascitic fluid showed forms of the colon bacillus, but the ascitic fluid kept in the incubator presented only pure cultures of an extremely minute diplococcus. After keeping for three weeks, subcultures upon agar made from the ascitic fluid no longer gave the typical colon form; instead of this a modified form was obtained; the individuals remained relatively small and very short. (*Vide* Fig. 5, Plate I.) Only after prolonged subculture and successive inoculation from 1 per cent. glucose broth did the forms become slightly larger and develop into a stumpy diplo-bacillus smaller than the typical colon. What is more, they did not induce fermentation of glucose or dextrose broth or cause the indol reaction. It must be pointed out that by this process of successive cultivation through glucose broth the form which was a characteristic diplococcus had become converted into a small bacillus arranged as a diplo-bacillus, and this stained homogeneously.

Upon passage through three guinea-pigs (the guinea-pigs being

killed from twelve to twenty-four hours after intraperitoneal inoculation) and growth upon 2.5 per cent. lactose broth the form has become still larger and more typical, but we still fail to obtain gas production. (*Vide* Fig. 6, Plate I.)

Within the last few days we have again obtained this diplococcus form from the human body. The patient, under Dr. Garrow, in the surgical wards of the Royal Victoria Hospital, suffering from marked biliary crises, was operated upon, in the expectation of finding a condition of cholecystitis with gallstones. Upon opening the abdomen a small amount of fluid presented, and a platinum loop of this was smeared upon agar-agar—this remained sterile—and immediately about a drachm of the fluid was collected, under strict aseptic precautions, in a sterile flask and brought over to the pathological laboratory. Here this was added to about an equal quantity of sterilized broth and placed in the incubator.

Upon continuing the operation the gall-bladder and ducts were found pervious; there was, however, a condition of perihepatitis, with subacute peritonitis affecting the upper half at least of the abdominal cavity, and with this was associated some thickening of the great omentum.

Upon examining the above-mentioned broth culture after twenty-four hours, Dr. Brown, the resident surgeon, found that it contained a pure culture of minute diplococci, and immediately called our attention to it. In the features of this growth upon various media this form has so far been found to resemble the minute diplococci already mentioned as obtained from the case of cirrhosis, though the growth is slightly more active and free. Passage through guinea-pigs and lactose broth has resulted in the development of a form identical with that just mentioned. (*Vide* Fig. 7, Plate I.)

There is very slow development of turbidity in ordinary broth, rather more rapid in glucose broth, but absence of any sign of fermentation. The growths upon the surface of agar in both were at first singularly fine, so that they resembled closely those of the streptococcus pyogenes, though possibly more transparent than the latter. Upon potato the growth was invisible; upon blood-serum the colonies were also very fine, and were of an opaque white fading on to a yellow tinge. Upon gelatin there was slow growth without liquefaction, while litmus-milk was decolorized until it became almost perfectly

white; then slowly, in the course of the fifth day or so, a fine pink color was developed in the medium; the milk is coagulated at the end of a week. Growth upon broth was definite, but not abundant, and was associated with singularly little turbidity, a white somewhat stringy precipitate being slowly formed. In the fermentation tube the open limb became opalescent or moderately turbid in the course of forty-eight hours, the closed limb remained perfectly clear, and, in addition, in neither glucose nor in lactose broth was there any production of gas; further, there was and is no indol reaction, and if turbidity be present it is still singularly slight.

It is unnecessary here to describe all the methods that we have employed in order to cause these forms to revert to type. Briefly, we may say that we have obtained the greatest change by culture for twenty-four hours upon broth rendered 1.5° acid, according to the method recommended by the Committee of Bacteriologists, to which 2.5 per cent. of lactose has been added. In this medium, already at the end of twenty-four hours, there is abundant growth and well-developed turbidity, and the individual forms are relatively large and ovoid, frequently arranged as stumpy bacilli. (*Vide* Figs. 6 and 7, Plate I.)

When this form is inoculated into the guinea-pig intraperitoneally and cultures made from the peritoneal fluid at the end of nine hours, both upon agar and glucose broth, growth upon glucose broth in the fermentation tube is much more active than before inoculation; and, whereas, previous to inoculation, only the open end of the tube had been rendered opalescent, now there is turbidity throughout both tubes. As already stated, after passage through three guinea-pigs and growth on this medium the form produced is undistinguishable from the normal colon bacillus.

It is possible that this remarkable and somewhat persistent diplococcoid form, obtained both from the bile of the inoculated guinea-pig and from the ascitic and peritoneal fluids, has become attenuated during its stay in the body, and that in the case of the bile, for example, during the passage through the liver, the colon bacilli have been markedly modified. We have taken sterilized human bile and added to this a minute quantity of a stock culture of the colon bacillus, and have not been able to obtain in the bile the diplococcoid form alone, although it is true that diplococcoid forms have been relatively abundant.

Here it is interesting to note a point which we again find observed by Rodet—namely, that the human bile has a distinct inhibitory effect upon the multiplication of the colon bacillus. Bile to which a minute drop of a twenty-four-hour old culture had been added remained to all appearances perfectly clear, and apparently no growth had occurred during four days; but when a drop of this bile was added to about 10 c.cm. of slightly alkaline broth and placed in the incubator, that broth rapidly became turbid, and there was most abundant development of the bacilli. We are making further observations upon this modification of the bacillus by growth in bile. This, however, may be said at the present time, that possibly the existence of bacteria in the bile may easily be overlooked when the ordinary methods of culture upon solid media are employed, the concentrated bile inhibiting their growth.

One of us (M. E. A.) has already found that human bile (three cases), which was apparently sterile when streaked upon agar-agar, gave abundant cultures of the colon bacillus when a small drop was added to about 10 c.cm. of glucose broth.

CONCLUSIONS. Thus far, then, our observations upon the colon bacillus grown outside the body have led us to the following conclusions:

1. The short form of the normal colon bacillus cultivated upon the ordinary bacteriological media frequently presents polar staining, the appearance given being that of two rounded bodies, staining more deeply than the rest of the bacillus, lying in and united by less deeply staining material.

2. In the more filamentous form a succession of these more deeply staining bodies is at times to be recognized.

3. Growth outside the body under relatively unfavorable conditions renders the polar staining more prominent, so that the shorter forms may closely resemble diplococci, and the filamentous forms show a common unstained or lightly-staining sheath, in which is to be made out a succession of minute dots in pairs and of somewhat larger single ovoid dots.

4. We have so far been unable by modifying the reactions of ordinary media, and by continued growth at a high temperature (46°), to produce cultures in which the diplococcoid form alone has been present, although by these means we have gained cultures in which this form has predominated.

5. On the other hand, certain body fluids sown naturally, if we may so term it, with the colon bacillus—*i. e.*, the ascitic and peritoneal fluids from a case of hepatic cirrhosis and of peritonitis respectively, and the bile of a guinea-pig inoculated with (? an attenuated form of) the colon bacillus have yielded us diplococcoid growths so modified that we have not so far been able to cause them to revert completely to type.

6. It has been by the prolonged action of these fluids that these races of the colon bacillus have been produced; cultures made from them immediately after removal from the body have yielded us, either immediately or after one or two transfers, typical cultures of the colon bacillus. Where the fluid has been kept from ten to twenty days the modified diplococcoid form has been produced.

7. The slight but definite inhibitory action of bile upon the growth of the colon bacillus is shown in two ways: (*a*) Smear cultures of bile upon agar may remain sterile, whereas the same bile added to ordinary peptone broth may be the seat of active growth. (*b*) Similar bile kept for several days in the incubator remains clear and shows singularly little evidence of growth within it, though subcultures from this yield fairly numerous colonies of a modified diplococcoid form of the bacillus.

8. The ascitic fluid from a case of hepatic cirrhosis was found to possess similar properties of modifying the colon bacillus and inhibiting its growth.

9. These modified colon bacilli are relatively minute, assume a diplococcoid form, are non-motile, form pin-point colonies upon agar-agar, cause but slight turbidity in broth and an almost invisible growth upon potato; act but slowly upon litmus-milk, have lost the power of fermenting glucose, lactose, and dextrose broth, and do not develop the indol reaction.

PART II.

ON THE DIPLOCOCCUS-LIKE MODIFICATION OF THE COLON BACILLUS IN THE TISSUES.

Taking a series of four young rabbits weighing from 225 to 305 grammes, we inoculated into the marginal vein of each 0.75 c.cm. of a twenty-four-hour growth of the colon bacillus, and killed the animals

at intervals of fifteen minutes, thirty minutes, one and two hours. The various organs were immediately placed in formol-Müller, and were subsequently cut in celloidin and paraffin, the sections being stained by carbol thionin.

Our attention was at first especially directed to the liver. Here already in the animal killed at fifteen minutes after intravenous inoculation a definite series of changes was seen to have occurred. (*Vide* Figs. 13 and 14, Plate II.) In the bloodvessels of the liver free bacilli of normal size and appearance were occasionally to be observed, but already bacilli could be recognized within the leucocytes in the blood-stream. (*Vide* Fig. 13.) The number of these leucocytes was not excessive, but each contained a relatively large number of bacilli. In addition, already the endothelium lining the vessels was seen to be very prominent; here and there these cells contained a fairly large number of bacilli.

In thirty minutes the number of bacilli in the endothelium cells and the number of endothelial cells containing bacilli were markedly increased. The bacilli, situated within the endothelial cells, already show strongly marked differences from those free in the blood-stream. The latter were of normal length and thickness, and took on a homogeneous stain. Those within the endothelial cells were short and stumpy, sometimes almost coccus-like. The appearance given is that of primitive bacilli having been broken up into shorter lengths.

In the rabbit killed at the end of one hour the number of bacilli seen in the blood-stream was distinctly less, but there was a further increase of those in the endothelial cells. Occasionally, in the endothelial cells relatively large bacilli could be seen, but the majority of forms were, as in previous specimens, very short and stumpy, and the impression gained by a study of the sections is that the bacillus is taken up in the long form and subsequently broken up into shorter sections. So far no well-stained bacilli could be seen in the liver cells. Already in the endothelial cells certain of these stumpy forms had the appearance of diplococci of fair size.

In the liver of the rabbit killed at two hours after inoculation the same appearances were to be made out as those seen in the rabbit of one hour—namely, the presence of short and stumpy bacilli in the endothelial cells: we were of the opinion that a larger proportion of these had the appearance of diplococci than in the previous sections.

In several places between the liver cells, as indeed also in sections taken at an earlier period, there were to be made out hyaline masses, apparently situated within the vessels, which hyaline masses contained numerous bacilli. We have found some little difficulty in coming to a conclusion as to the nature of these masses; the large ones would seem certainly to be hyaline thrombi, but in the smaller ones it was often difficult to make quite certain whether we were not dealing with some phenomenon in connection with the endothelial cells; for very frequently a nucleus of endothelial type was in close connection with these smaller hyaline masses. We could not absolutely leave out of account the possibility that we were dealing with very greatly swollen endothelial cells.

Up to this point we were unable to recognize in any of the sections of this series indications that the bacilli had been taken up by the liver cells. But in a rabbit killed four hours after inoculation we came across great numbers of extremely minute brownish shadows definitely within the hepatic parenchyma. (*Vide* Fig. 15.) We have been wholly unable to stain these little bodies, and, indeed, only by very careful examination with the $\frac{1}{18}$ immersion lens have we been able to see them distinctly; but with this magnification there they most certainly are, and the more carefully they are studied the more clearly they are seen to be present in general as extraordinary minute little brownish diplococci, at times showing a halo around them. And the more one has studied these appearances the more it seems likely that this apparent halo indicates that these small bodies lie in vacuoles, although in part also the appearance may be due to the existence of an unstained sheath or body-substance.

Evidently, judging by the sections from this stage of the inoculation disease, not only are the bacilli taken up in large numbers into the liver cells, but being taken up they undergo rapid digestion and destruction, so that they can no longer be stained by the ordinary methods, and what we see are essentially the shadows of the bacilli. We have attempted to make out the stages by which the bacilli pass from the endothelium into the liver cells, but so far without great success. Here and there in sections of the two-hour rabbit we have been able to make out that the endothelium appeared to be raised from the underlying cells, and on the inner side of this endothelium very rarely we could see in the spaces between the endothelium and

cell well-stained coccus or diplococcus-like bodies. We are, however, unwilling to dwell too strongly upon these appearances, inasmuch as the endothelial cells showing these features were crowded with bacteria, and we could not exclude the possibility that in the process of preparation the cells might have become slightly dislodged, and that the appearance of the bacilli apparently outside the main body of the cells might be due to their presence in a slightly different plane.

Taking next well-developed rabbits similarly inoculated and killed at the end of twenty-four hours, we have found in them the presence of bacilli in the endothelial cells, while the brown shadows, as we may term them, have been present in enormous numbers in the liver cells.

Thus far, then, from what we have said, it would appear evident that when the colon bacillus enters into the circulation it is liable to be taken up rapidly by the endothelium lining the hepatic vessels, and in this process undergoes division into smaller segments, so that in the main one meets with stumpy forms in these cells, forms which still stain well, although often showing a tendency toward a diplococcoid appearance. Following up this within four hours these bacilli are discharged by the endothelial cells, and are by some means or other taken up by the hepatic cells and rapidly destroyed, so that it is only by careful examination that minute coccus or diplococcus-like bodies are discovered within the liver cells.

It is interesting to note that upon examining a film of the bile taken from inoculated animals at the end of twenty-four hours one can by careful preparation recognize in it these very minute diplococcus-like bodies. To see them it is necessary to make a very fine film, treat with weak acetic acid, wash, and then stain with dilute carbol-fuchsin, and examine under the highest power. It would, therefore, seem evident that the liver cells are capable of discharging these modified and destroyed bacilli into the bile capillaries.

But we now come to certain great difficulties in connection with the statements here made. In the first place, making a large series of control observations upon the livers of apparently normal adult rabbits we have frequently come across these same diplococcus-like bodies, and in four instances in relatively very great numbers. Indeed, these diplococcus-like bodies would seem to be very frequently present, more often present than absent from the rabbit's liver.

To obviate this difficulty, it seemed to us that we might obtain more decisive results by employing very young rabbits from three to six weeks old. In our control sections of the livers of these very young rabbits we have found that the diplococci appear to be absent. Upon making a like series of inoculations into these very young rabbits, and killing at two, four, and twenty-four hours, we hoped definitely to settle the question. But here, at first, we had wholly negative results. By our routine methods of staining we were unable to detect any bacteria within the cells, even when we employed sections that had been cut in paraffin. So opposed to all our previous results and conclusions did these appear that for a time we were on the point of relinquishing this paper. It is possible that either the carbol-thionin used by us for the experiments was defective or our technique modified in some slight degree, for at the best the carbol-thionin method does at times show itself wanting. But our failure was so constant that we hardly believed that this explanation would suffice.

Now we have attempted to stain other sections from the same blocks by other methods, and we eventually found that staining for half an hour with Loeffler's methylene-blue, washing with tepid water, and then passing through absolute alcohol and zylol, we obtained sections in which the tissue is relatively faintly stained and in which we are able to detect within the cells peculiar small diplococci having the faintest brown tinge. These were obtained from the livers of animals which had been inoculated two and four hours before death. Our failure to recognize these bodies is in fact due to their minute size and their very faint stain. We have examined control livers also from young animals by the same methods with negative results.

It would seem clear to us that the rate at which the colon bacilli are taken up and destroyed in the liver varies to some extent in different animals according to the condition of the tissues and the virulence of the microbe. It is to be noted that the culture employed in this latter series was from the same stock as that employed previously—a stock which had been grown outside the body for an additional six months. And here we may notice that the most powerful staining diplococci, and also those having the deepest brown tinge, were in the livers of rabbits dying from three to four weeks after inoculation, as again in certain of our control animals. Our experimental animals, which had been kept alive at the most for twenty-four hours, have

yielded us only diplococcus forms, showing but a delicate brown staining within the cells.

While we were in doubt with regard to this second series of livers, it seemed to us well to study another excretory organ not in connection with the portal circulation. Examining the kidneys of several control rabbits, we have in no case been able to find the diplococcus forms present within the organ in the great numbers in which we have come across them in the livers in the same animals. We have met with occasional diplococci within the cells of the convoluted tubules, but these have been rare. We have found that the examination of the kidneys for these modified colon bacilli has been a matter of considerable difficulty. Undoubtedly they are taken up by the cells of the convoluted tubules. Of this we have abundant evidence, and occasionally we have come across well-staining diplococcus-like forms in the outer portion of the kidney cells, but the diplococcus forms appear to be destroyed with great rapidity, and in the process of destruction do not assume the brownish tinge already referred to in connection with the liver; thus it has been a matter of extreme difficulty to trace them. We have, however, seen them in great numbers in the cells of the convoluted tubules of the rabbit two hours after inoculation (*vide* Fig. 11, Plate I.) and again at twenty-four hours, in this latter the number being greater. Here, also, in the cells of the tubules in very thin sections we have come across numerous minute vacuoles of an elongated oval shape, often slightly dented in the middle; and within these we have at times been able to distinguish two very minute dots, evidently the very final indication of the disappearing and destroyed bacillus. (*Vide* b. and c., Fig. 11.) Independently, Dr. A. G. Nicholls⁷ has studied the kidneys of the animals inoculated by us and has met with these diplococcus forms, fully confirming what we here state.

CONCLUSIONS. Our observations, therefore, upon the whole would lead us to the following conclusions:

1. That the colon bacillus injected into the circulation is rapidly taken up both by the liver and the kidney.
2. That within fifteen minutes after inoculation some bacilli are already ingested by the endothelial cells in the liver, this process of ingestion continuing until these cells are full of bacilli.
3. That in this process of ingestion the bacilli are broken up into

shorter lengths, and that these short stumpy bacillary forms may—already within the endothelial cells—present themselves as two deeply staining dots, and may thus resemble diplococci.

4. That already in two hours the modified bacilli may be discharged outwardly from the endothelial cells and be taken up by the underlying liver cells.

5. The exact stages of this discharge we have been unable to follow. In the liver cells the modified bacilli are to be recognized as small diplococci of a size varying from that equal to the diplococci seen in the endothelial cells down to points of extreme tenuity. Evidently these forms are undergoing destruction.

In the first place, they lose their power of staining; in the second, if the destruction is not too rapid, they assume a brownish tinge. What is the causation of this brownish tinge we have not yet determined, but it is to be made out in the unstained sections, and our studies upon the human liver indicate to us that not a little of the fine pigmentation common in liver cells is brought about by the existence in these cells of these minute elements of bacterial destruction.

During this process of destruction the modified bacilli lie in digestive vacuoles, and the frequent appearance of the halo around these forms is in a great part due to the existence of the vacuole. We have occasionally been able to make out what appear to be these vacuoles in the liver cells without the evidence of the contained microbe, that having been apparently entirely digested. (We have seen the same appearance also in peritoneal leucocytes nine hours after intraperitoneal inoculation with modified colon bacilli.) (*Vide* Fig. 10, Plate I.)

6. In the kidney the same process is at work. We have recognized the diplococcus form within the cells at the expiration of two hours after inoculation, and have also seen the vacuoles within the cells and convoluted tubules, and there have occasionally met with two dots just visible, being final indications of the process of digestion of the bacillus.

We sincerely hope that others will repeat and confirm these observations, though to those repeating them we would point out that it is absolutely essential to employ higher powers than those ordinarily used for bacteriological investigations, while the finest sections are requisite to give clear results. Very careful technique in the matter

of intensive staining and of decolorizing of the tissues is also an essential. Unless these points are attended to our frequent difficulties in forging the chain of evidence here brought forward will certainly present themselves, and, without great patience, we cannot expect others forthwith to corroborate our results. We are prepared, that is, to find these results called in question; but after many months, and on the part of one of us many years, puzzling over these peculiar pigmented bodies, seen more especially in the liver, we do not see what other conclusion to reach. Here we may say that we are prepared to find bacteria other than the colon when taken up at the cells of the liver and kidney assume very similar forms. Indeed, we already have evidence of this in connection with the typhoid bacillus.

If the above conclusions are correct, it is clear—judging from what we have said concerning the appearances seen in many normal livers of rabbits, and seen also, we may add, in the human liver—that the liver as an organ possesses the most important function of taking up and destroying bacteria, more especially the colon bacilli, which have gained admission through the portal blood, while the kidney possesses a like power of rapidly destroying bacteria circulating in the general systemic blood.

As our paper is more especially upon this diplococcoid form of the colon bacillus and its modifications within the body, we will not here dwell upon this subject, especially as one of us has already called attention to this conclusion elsewhere.¹

We purpose, if possible, making a series of observations upon those conditions which lead to the taking up of bacteria from the intestines and upon their course through the blood, and again through the lymphatic system.

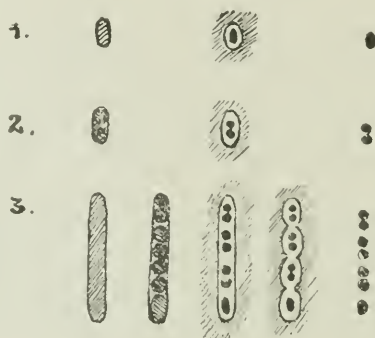
Finally, a few words regarding the structure of the colon bacillus to be deduced from the above observations. It is difficult to arrive at any other conclusion than that the type colon bacillus is a stumpy bacterium with rounded ends. This bacillus consists of at least two parts—one which takes deeply the stain, the other relatively non-staining. Under ordinary conditions of free and rapid growth these are not to be distinguished from each other; under other conditions, more especially those of difficult growth, the chromatin or staining portion tends to be aggregated along the long axis of the bacillus, most characteristically in the stumpy bacillary form, as two rounded bodies,

and thus the appearance is given of a diplococcus, a capsulated diplococcus, the apparent capsule being the non-staining body-substance.

Where the bacillus is of the large or filamentous type our observations would seem to show us that the filament is capable of being broken up with a certain amount of ease—*e. g.*, in the endothelial cells—into its component stumpy or bacterial forms, each of these being either a single oval deeply staining body, or, as above mentioned, two rounded staining bodies, so that it assumes the diplococcus form. The size of these chromatin bodies varies, as would be the case were the chromatin capable of varying degrees of concentration.

Judging from what is observed within the hepatic and renal cells, these chromatin bodies consist, at least, of a basal non-staining material and a chromatin, for the power of staining (with aniline dyes) may completely disappear, and, nevertheless, a substance is left behind still capable of recognition as a minute diplococcus, unstained by ordinary reagents, but, within the liver cell, capable of assuming a brownish tint. From the appearance within the body cells this central substance is obviously more resistant than the remainder of the bacillus. It is the last part of the bacillus to be destroyed; indeed, these diplococcus-like shadows of bacilli may accumulate more especially within the liver cells, the mesenteric and retroperitoneal glands.

We will not here discuss the recent work upon the existence of nuclei or nuclear material in the schizomycetes. It is, however, impossible not to be struck by the analogy in structure between the colon bacillus as here described and nucleated cells in which nuclear division precedes cell division. For we have encountered the following forms:



This paper being already longer than we had intended to present, we have omitted any consideration or criticism of the observations by Gärtner,⁸ Klein,⁹ Thiercelin,¹⁰ and many others which show a recognition or failure of recognition of the existence of this diplococcoid form of the colon bacillus, and very frequently a tendency to mistake the diplococcoid and the encapsulated form of the colon bacillus for an entirely different species. This subject will be discussed at greater length by one of us (M. E. A.) in a separate article.

EXPLANATION OF PLATES.

The figures of bacteria have all been drawn (by J. G. A.) under the same magnification—i. e., Reichert $\frac{1}{13}$ in. immersion, ocular 4—by means of a Zeiss camera lucida, latest pattern. All were subjected to the same process of staining: Ziehl-Neelsen carbol-fuchsin diluted with 50 per cent. of water. They were relatively deeply stained and then decolorized in water containing a minute proportion of acetic acid—about one drop of glacial acetic acid to the ounce of water. The drawings of sections were made under the same conditions, with the exception of Fig. 11, Plate I., which is a tracing from a photograph.

PLATE I.

FIG. 1.—Growth originating from bile of guinea-pig which died twenty-four days after intraperitoneal inoculation with 10 c.cm. of ascitic fluid from case of hepatic cirrhosis; forty-eight hours' growth in alkaline peptone broth inoculated from forty-eight hours' culture upon agar-agar, which in its turn had been seeded from a pipette of the guinea-pig's bile removed a few minutes after death and kept for eighteen hours at 37°.

FIG. 2.—From film made from the ascitic fluid of the above case of hepatic cirrhosis left in sterilized flask at 37° for seventeen days.

FIG. 3.—From first broth culture, forty-eight hours old, made from the above ascitic fluid. Note minute ovoids as well as diplococci.

FIG. 4.—From forty-eight-hour culture upon Loeffler's blood-serum made direct from the above ascitic fluid: forms a shade larger than those from broth, with slight tendency to be arranged in short chains.

FIG. 5.—The same micro-organism after repeated transfer upon agar agar during six months: individuals much larger, although still relatively small, with short bacillary, diplo-bacillary, and diplococcoid forms.

FIG. 6.—The same after transfer through three guinea-pigs, followed by three successive transfers through 2.5 per cent. lactose broth: the bacillus now is morphologically indistinguishable from the forms seen in Fig. 1 and (save in absence of flagella) from the ordinary colon bacillus.

FIG. 7.—Microbe isolated from peritoneal exudate in case of peritonitis with perihepatitis after parallel passage through three guinea-pigs and transfer through lactose broth. The microbe, upon first isolation, was a minute diplococcus: it will be seen to be undistinguishable from that shown in Fig. 6; culturally, it was identical.

FIG. 8.—To show arrangement of diplococci and ovoids in filamentous form of a colon bacillus isolated from the spleen in a case of progressive hepatic cirrhosis. From a colony on an agar plate kept five days under unfavorable conditions (vide *British Medical Journal*, October 22, 1898).

FIG. 9.—From film made from the bile of a rabbit killed seven hours after intravenous inoculation with the colon bacillus; the bile was kept fourteen days in pipette before examination. Note variety of forms: rare short bacilli and diplo-bacilli with slight capsule, diplococci with well-marked capsules, minute diplococci devoid of capsule (? destroyed).

FIG. 10.—Cells from peritoneal fluid of guinea-pig killed nine hours after intraperitoneal inoculation with a forty-eight-hour broth culture of form shown in Fig. 5—i. e., from agar cultures derived from the ascitic fluid from a case of cirrhosis. Stained with carbol-thionin,

Reichert $\frac{1}{18}$ in. immersion, ocular 4, drawn under a Zeiss camera lucida. *a.* Deeply staining bacterial and diplococcoid forms. *b.* Attenuated diplococcoids in large vacuoles. *c.* Still further attenuated diplococcoids. *d.* Vacuoles void of contents.

FIG. 11.—From section of convoluted tubules of kidney of young rabbit killed two hours after intravenous inoculation with pure culture of bacillus coli. Tracing from photograph under Zeiss $\frac{1}{18}$ in. immersion, compared with original section (the photograph not being perfect). *a.* Deeply staining diplococcoid form just within cell of tubule. *b.* Attenuated diplococcoid form in vacuole. *c.* Empty vacuoles of oval shape.

FIG. 12.—To compare with Fig. 1. From film of bile from guinea-pig kept in pipette and placed in incubator at 37° for eighteen hours. A drop of this same bile passed through broth and agar gave the form shown in Fig. 1. Note presence of ovoids, diplococcoids, and diplococci.

PLATE II.

FIG. 13.—Section of liver of rabbit killed fifteen minutes after intravenous inoculation with 0.5 c.c. of forty-eight-hour broth culture of *B. coli*; carbolic thionin; Zeiss camera lucida; $\frac{1}{18}$ oil immersion.

a. Marked swelling and enlargement of endothelial cell with ingestion of bacilli, which have become short and stumpy.

b. Free bacilli, remaining long.

c. Leucocytes containing bacilli.

FIG. 14.—Section of liver of rabbit killed fifteen minutes after intravenous inoculation with 0.5 c.c. of forty-eight-hour culture of *B. coli*; carbolic thionin; Zeiss camera lucida; $\frac{1}{18}$ oil immersion lens.

a. Bacillus free in capillary.

b. Endothelial cell, much swollen and containing several bacilli, both stumpy and tending to assume diplococcoid form.

PLATE III.

FIG. 15.—Section of liver of rabbit killed four hours after intravenous inoculation with 0.5 c.c. of forty-eight-hour culture of *B. coli*; carbolic thionin; Zeiss camera lucida; $\frac{1}{18}$ oil immersion lens.

Abundant very minute diplococcoid forms in liver cells, part of which only are shown.

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PLATE I

Adami—Diplococcoid Form of the Colon Bacillus.

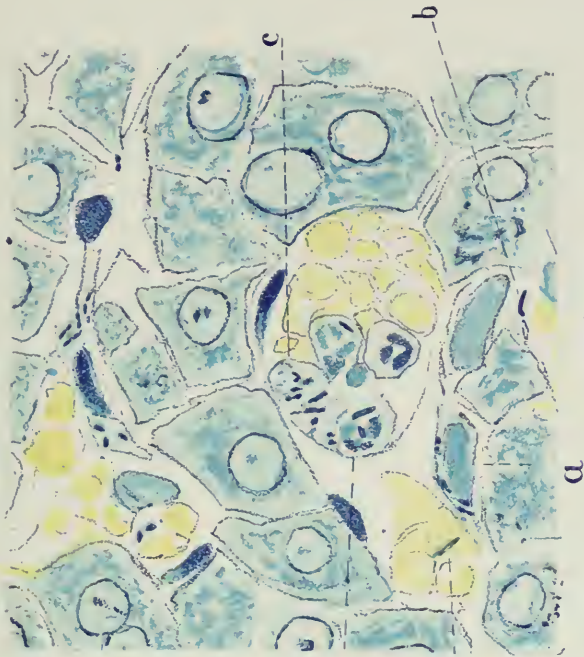


FIG. 13.

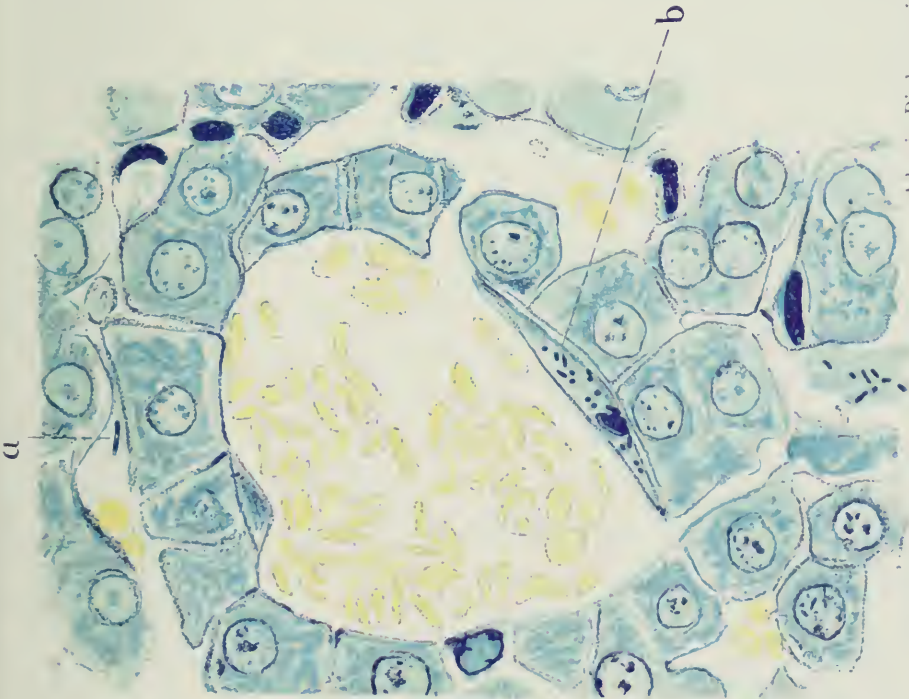


FIG. 14.

PLATE II
Adami—Diplococcoid Form of the Colon Bacillus.

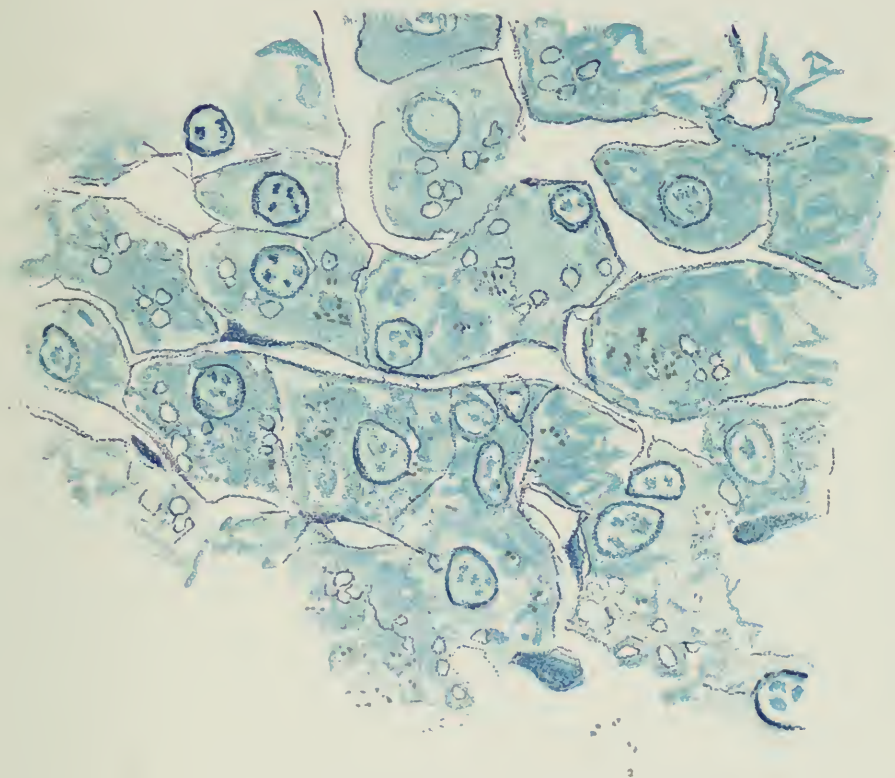


FIG. 15.

PLATE III

Adami—Diplococcoid Form of the Colon Bacillus.

DISCUSSION.

DR. WELCH: Dr. Adami's observations are of great interest and most suggestive, although their interpretation is not without difficulties. The colon bacillus is undoubtedly encountered very often in bacteriological examinations at autopsies, but only in a relatively small number of these cases does its presence seem to be of any pathogenic significance. Blachstein's experiments showed that colon bacilli injected into the circulation not infrequently cause necrotic foci in the liver, where they may survive a long time, and that the bacilli are often eliminated by the bile. We found weeks, and even months, after the injection the bacilli in the bile, in which they had induced certain changes suggestive of the causation of gallstone by biliary infections.

Dr. Adami and his coworkers have brought forward new observations in support of the opinion that bacteria are very frequently absorbed from the intestinal tract, and that certain organs, particularly the liver, dispose of these bacteria by intracellular digestion. Under the designation of the "diplococcus form" of the colon bacillus, Dr. Adami describes conditions and appearances of this bacillus, which I judge not to be identical, and it is possible that some better designation might be used. I confess to a feeling of some uncertainty as to the exact interpretation of the small coccoid bodies demonstrated by Dr. Adami in the hepatic and other cells, but I do not question that he has brought forward to-day and in his previous papers strong arguments in favor of his conclusion that they are bacterial forms or are derived from such forms.

DR. ADAMI: This subject opens up to a large field, and there is yet so much to be done, in order to establish the fundamental points in connection with the reaction of the tissue cells on the colon bacillus under normal and pathological conditions, that I fully accede to Dr. Welch's statement, that this matter must at the present time be received tentatively. I cannot expect others who have not worked at the subject to accept freely and immediately the observations which I have just recorded; indeed, the observations upon this very minute form require very great care. Thus I recognize that it may be some years yet before the statements here made receive general acceptance, and I am myself prepared to spend some years yet in working at the subject. I have, however, seen sufficient during my work this last year to feel assured that others must eventually see that my observations are fundamentally correct.

